U.S. Department of Energy, Office of Science
Environmental Remediation Sciences Division (ERSD)
FY08 First Quarter Performance Measure

Introduction

In the first quarter of fiscal year 2008, the Rifle IFC completed the biostimulation phase of the first Rifle IFC field experiment. Previous work, planning, execution, and analysis of this experiment and related column experiments at Princeton and UC Berkeley provide the basis for identifying critical *in situ* redox reactions. The ERSD FY08 First Quarter Performance Measure entitled "Provide a report documenting the approach to identify critical redox reactions in the subsurface during stimulated uranium biotransformation" was successfully met by these activities.

Background

A promising approach to remediating redox-sensitive contaminant metals in subsurface environments is microbial reduction of the oxidized, mobile forms to sparingly soluble, reduced forms (DOE, 2006). Development of this approach from bottle incubations to field experiments has been a major success story of the U.S. Department of Energy (DOE) Environmental Research Science Program (ERSP; formerly NABIR). After the initial pure culture studies on this process, pioneering work in developing this approach was first conducted with subsurface sediments collected from three Uranium Mill Tailings Remedial Action (UMTRA) sites. Field-scale electron donor amendment experiments followed at the ERSP UMTRA study site in Rifle, Colorado. Analogous approaches have now been successfully employed at other DOE sites such as the Field Research Center at Oak Ridge National Laboratory (ORNL; (Roden and Scheibe, 2005)) and near the 100-H Area on the Hanford Site (Hazen et al., 2004).

The field study site at Rifle (Figures 1, 2) has proven to be ideal for conducting *in situ* biostimulation experiments for removal of U(VI) from groundwater. Shallow depth to water (3-4 m), thin saturated thickness (~2.5 m), and impermeable lower boundary create a readily accessible, well-defined groundwater flow system that makes it inexpensive to monitor microbial and geochemical processes and to assess the progress of *in situ* uranium bioremediation in real time. The site exhibits typical geochemical effects on uranium mobility (e.g. bicarbonate control on U(VI) sorption) and is also readily biostimulated, indicating that it offers a balance among competing biotic and abiotic processes, making it feasible to determine which processes dominate. Studies at the Rifle site have already demonstrated that the analysis of transcript levels for key metabolic, regulatory, and respiratory genes can provide unprecedented insight into the factors controlling the rates of metabolism and can identify the most important environmental factors that can be modified *via* groundwater amendments to adjust microbial metabolism to optimize the bioremediation process (Holmes et al., 2005). The emerging science of proteogenomics (Banfield et al., 2005) also shows promise in preliminary data collected from Rifle site sediments from an *in situ* biostimulated zone. The novel techniques being developed at the Rifle site for monitoring the *in situ* metabolic state of microorganisms and their rates of respiration will be applicable to the study of bioremediation of a wide range of subsurface contaminants, including chlorinated solvents, at many DOE sites.

In addition to the applicability of their results to a wide diversity of DOE contaminated subsurface sites, investigations at the Rifle site offer the possibility of answering specific questions of immediate concern to the stewardship of UMTRA sites. The current approach for modeling natural attenuation of U(VI) at UMTRA sites is to use a site-specific distribution coefficient that characterizes the partitioning of U(VI) between groundwater and sediment combined with a groundwater flux calculation. Regulatory requirement is for U(VI) concentration to fall beneath the applicable standard (0.044 mg/l for inactive sites) within 100 years. For some UMTRA sites, U(VI) concentrations are not expected to fall below the standard for time spans

close to 100 years, but if the naturally occurring microbial community can be stimulated to reduce U(VI), then U(VI) concentrations could fall below compliance levels in shorter times. Understanding and optimizing the factors controlling U(VI) bioreduction in the context of quantitatively mechanistic reactive transport models developed in this project will provide a scientifically defensible basis for DOE-LM to estimate future concentrations of U(VI) in alluvial sedimentary aquifers. Of key interest is to develop models that evaluate the critical redox processes controlling U transport and include realistic descriptions of the field-scale biogeochemical reaction network, including the role of evolving microbial populations, the competition between various terminal electron acceptors for the electron donors, the bioavailability of various Fe(III) sources, and a mechanistic description of U(VI) adsorption on hydroxide and biopolymer surfaces, taking into account the competition from other metals (principally Fe) and the tendency for U(VI) to form stable complexes in solution.
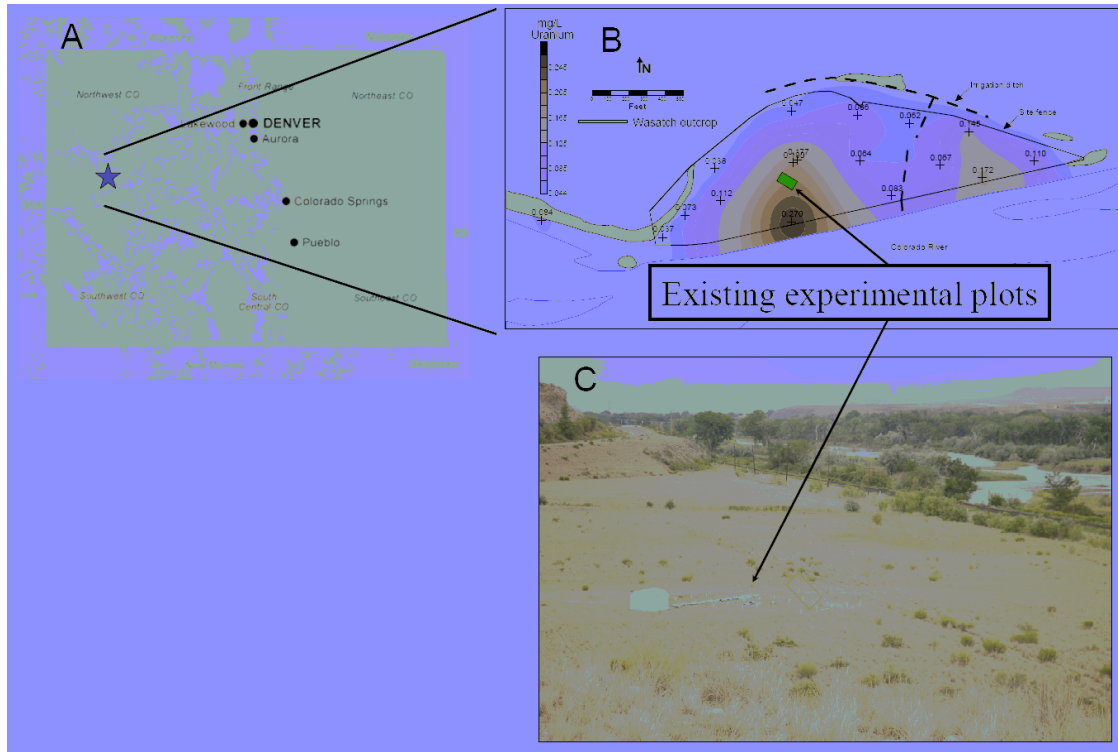
Figure 1. (A) Location of the proposed Rifle Field Site in Colorado. (B) Map of the site showing estimated U(VI) concentration in groundwater as of approximately 1998 and location of existing experimental plots on the site. (C) Photograph of the site looking east.
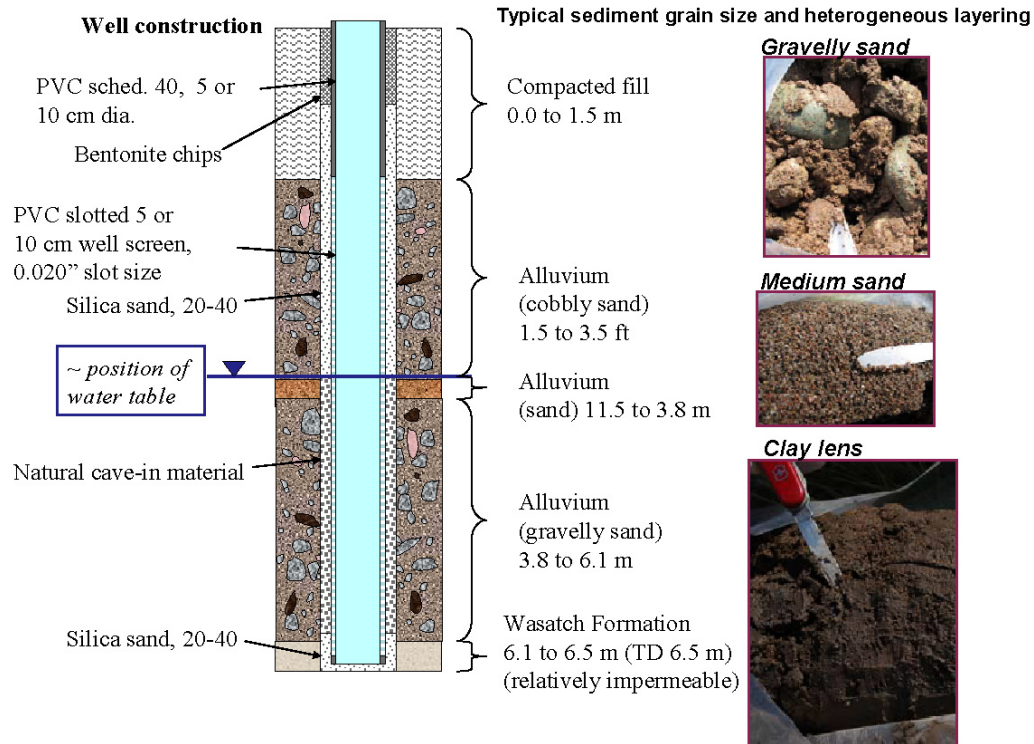
Figure 2. Stratigraphic Section and Lithologies of the Alluvial Aquifer at the Rifle Site

Identification of critical redox reactions in the subsurface during stimulated uranium biotransformation.

Preliminary research involving enrichment and pure culture analyses demonstrated that Fe(III)-reducing and some sulfate-reducing microorganisms can reduce the soluble oxidized form of uranium, U(VI), to relatively insoluble U(IV). Much of the early research focused on Fe(III)-reducing microorganisms, e.g., *Geobacter metallireducens* (Lovley and Phillips, 1992a, b; Lovley et al., 1991; Lovley et al., 1993). The insights gleaned from these studies were used to design acetate biostimulation experiments conducted from 2002 to 2005 to test the scale-up of the basic principle of enhanced uranium bioreduction to the field. During the acetate biostimulation at Rifle, U(VI) removal from groundwater was found to be highly efficient and correlated with the stimulated growth of a dominant iron-reducing population in the subsurface (Anderson et al., 2003). Rates of uranium reduction slowed as the microbial community shifted to a predominantly sulfate-reducing community. Subsequent to stopping acetate amendment, ongoing removal of U(VI) from groundwater has been documented for two additional field experiments suggesting that persistent redox reactivity may be occurring within the biostimulated zone in the absence of additional acetate. Both in-progress and proposed research will address this unexpected observation.

Field data suggest that the bioreduction of Fe(III) and U(VI) likely by the native *Geobacter* population continued at a lower rate after the onset of sulfate reduction. Recent laboratory-column biostimulation experiments with aquifer sediments from the Rifle site (but low sulfate water introduced to the column) have shown that a much larger amount of Fe(III) was reduced over a 200-day biostimulation experiment than what one would have predicted based on a 1 h 0.5 N HCL Fe(III) extraction, representing "bioavailable" Fe(III)(Komlos et al., 2006). In contrast, the transition to sulfate reduction at the field site (where higher sulfate concentrations prevail) occurred at a point where approximately one-half of the poorly crystalline Fe-hydroxide (as indicated by 0.5 N HCl extraction) had been used (Yabusaki et al., 2007). The triggering

mechanism in this redox transition is believed to be due to alterations in the reactive mineral surfaces. This interaction between iron and sulfate reduction and its effect on uranium bioimmobilization both during and after biostimulation needs further investigation.

Summary conceptual models and redox reactions

The studies at Rifle have led to the following conceptual model for field-scale biogeochemical redox reactions and sorption processes controlling U(VI) mobility in the subsurface at the Rifle site.

Conceptual model for the site under ambient conditions. Residual U(VI) in the vadose zone gradually moves to the water table *via* vertical recharge or when the water table rises. Once in the saturated zone, U(VI) is transported horizontally by groundwater flow. Concentrations are gradually reduced by dilution, adsorption, and presumably *in situ* bioreduction. The largely aerobic microbial population produces very low dissolved oxygen (DO) (ca. 0.2 mg/L) groundwater with high bicarbonate content. Uranium complexation with bicarbonate results in a low distribution coefficient (K_d) for U(VI) in the field (ca. 0.1 to 0.3 ml/g (DOE, 1999)), and the low DO and nitrate enables maintenance of a small population of anaerobic bacteria, including active metal-reducing microorganisms that may be responsible for reducing Fe(III) to Fe(II) and U(VI) to U(IV). The net effect of natural microbial activity in the system is to decrease the uranium concentration in groundwater in spite of the increased bicarbonate resulting from microbial activity. The redox status of Fe and the amount of DO and U(VI) in the system varies with time and depth (Anderson et al., 2003; Vrionis et al., 2005) and is temporally linked to the seasonal river stage in the Colorado River. DO may also change spatially, possibly increasing within a few 10's of meters from the river. Geochemical and hydrogeological heterogeneities exist in the flood plain on the scale of the experimental plots (< tens of m).

Current conceptual model for the site under biostimulated conditions. Field-scale biostimulation experiments at the Rifle site have shown that electron-donor (acetate) addition consistently promotes the growth of dissimilatory metal-reducing bacteria (DMRB) of the *Geobacter* family that are known to enzymatically reduce U(VI) to U(IV). With biostimulation, detectable biomass increases by more than 10-fold, and a significant percentage of that biomass is dominated by *Geobacter* and related *Desulfuromonas* and *Pelobacter* species. The reproducible, stimulated growth of DMRB correlating with the loss of U(VI) from the groundwater suggests that these organisms are responsible for the *in situ* reduction of U(VI). However, maintaining metal-reducing conditions *in situ* depends on the amount of Fe(III) available. After consumption of about one-half of the bioavailable Fe(III), represented by poorly crystalline hydroxide, the anaerobic community shifts to a system dominated by sulfate-reducing bacteria (SRB), where removal of U(VI) from solution is significantly less than under Fe-reducing conditions. This response contrasts with that observed at the Oak Ridge Field Research Center ((Wu et al., 2006); T. Scheibe, pers. Communication) where U(VI) loss increased during sulfate reduction. The marked differences in response suggest site-specific factors strongly influence biostimulation, confirming the need for a more mechanistic understanding.
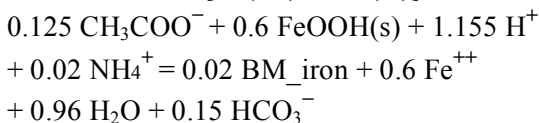
Removal of U(VI) in the post-biostimulation at the Rifle site lasts longer than expected based on the known sensitivity of U(IV) to oxidation (N'Guessan et al., 2006) Our understanding of why that occurs is still evolving and will be addressed by future experiments. Our best understanding to date is that reduction of U(VI) during biostimulation requires microbial mediation, but after cessation of electron donor amendment, the increasing removal of up to 85% of the influent U(VI) for more than one year may be the result of a complex interaction of a microbial community that maintains nitrate at low levels and abiotic reactions involving sorption of U(VI) by any Fe(III) oxides formed as the redox status of the system returns to more oxidizing conditions. Alternatively, prolonged activity of SRB and the resulting HS^- act to further reduce Fe in the clay-sized fraction. In-progress research (N'Guessan et al., 2006) indicates that preferential sorption of U(VI) by microbial cells or biopolymers may also be an important factor. Further

complication is suggested by recent research showing preferential sorption of U(VI) to Fe-containing octahedral-layer edge sites in phyllosilicates (Catalano and Brown, 2005; Catalano et al., 2006).
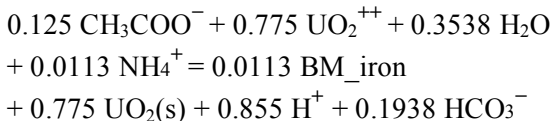
Finally, we do not know whether sulfate reduction is required for the long-lived *in situ* stability of U(VI). However, more rebound was observed in U(VI) after the shorter 2004 experiment, which had less sulfate reduction, indicating this might be the case. It is unknown, however, whether this is due, in part, to the O_2 buffering capacity of the Fe-sulfides formed during the sulfate reduction, or due to a mechanism like adsorption acting on newly formed Fe(III) oxides.

Although significant insight into the processes governing uranium bioreduction has been gained at the Rifle site, key factors and feedbacks controlling microbial dynamics, changes in mineralogy, and the transport rate and extent of U(VI) reduction during and after biostimulation need elucidation and quantification. The dominant reactions in the system are
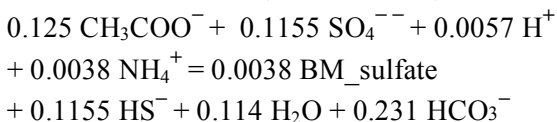
1. Fe-reduction [Fe(III) to Fe(II)]

$0.125\ CH_3COO^- + 0.6\ FeOOH(s) + 1.155\ H^+$
$+ 0.02\ NH_4^+ = 0.02\ BM\_iron + 0.6\ Fe^{++}$
$+ 0.96\ H_2O + 0.15\ HCO_3^-$

2. Uranium reduction [U(VI) to U(IV)]

$0.125\ CH_3COO^- + 0.775\ UO_2^{++} + 0.3538\ H_2O$
$+ 0.0113\ NH_4^+ = 0.0113\ BM\_iron$
$+ 0.775\ UO_2(s) + 0.855\ H^+ + 0.1938\ HCO_3^-$

3. Sulfate reduction (SO_4^{--} to HS^-)

$0.125\ CH_3COO^- + 0.1155\ SO_4^{--} + 0.0057\ H^+$
$+ 0.0038\ NH_4^+ = 0.0038\ BM\_sulfate$
$+ 0.1155\ HS^- + 0.114\ H_2O + 0.231\ HCO_3^-$

Where BM_iron and BM_sulfate are the biomass generated by the microbial growth during the particular reaction involving iron or sulfate reduction via oxidation of acetate (Yabusaki et al., 2007). A key goal for the U.S. Department of Energy is to gain a comprehensive and mechanistic understanding of the microbiology and geochemistry controlling uranium mobility at the field scale so that contaminant plumes of redox sensitive metals can be confidently modeled for long-term stewardship or remediated. Key processes that are not well understood relative to the above reactions include:

1) What forms of Fe(III) are actually involved in the reduction process?
2) How is reduced iron (Fe(II)) partitioned between the pore fluid and solid surfaces and what reactions occur between Fe(II) and solid phases under iron-reducing conditions?
3) Bioreduced U(IV) is known to be precipitated as nanoparticles (Suzuki et al., 2002). However, aggregation of these particles at solid surfaces could be a critical factor controlling their mobility and reactivity. As noted above, they may be stabilized by biomass and little is known about the potential importance of this process at the field scale.
4) Sulfate reduction can produce a variety of sulfide minerals ranging from amorphous FeS to pyrite (FeS_2). While FeS is known to be particularly reactive, the relationship between the form of sulfide minerals and uranium needs to be well understood to model the effect

of sulfate reduction and biomass of sulfate reducers on uranium sequestration in the subsurface.

Key remaining issues here are the reactions and mechanisms for stabilizing uranium *in situ* under either natural or biostimulated conditions. As noted above, one possibility is that groundwater flow conditions and redox buffering solids simply exclude dissolved oxygen or nitrate from reaching zones with reduced uranium. Another is that U(IV) or U(VI) is protected or stabilized by specific phases such as sulfide, magnetite, or biopolymers. Current research as part of the Rifle IFC is addressing these issues under both naturally occurring and biostimulated conditions.

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